

TITLE: MEANS AND METHODS FOR IDENTIFYING GENES AND  
PROTEINS INVOLVED IN THE PREVENTION AND/OR REPAIR  
OF A REPLICATION ERROR

Inventor: Tijsterman et al.

Docket No.: 2183-6201US

1/7

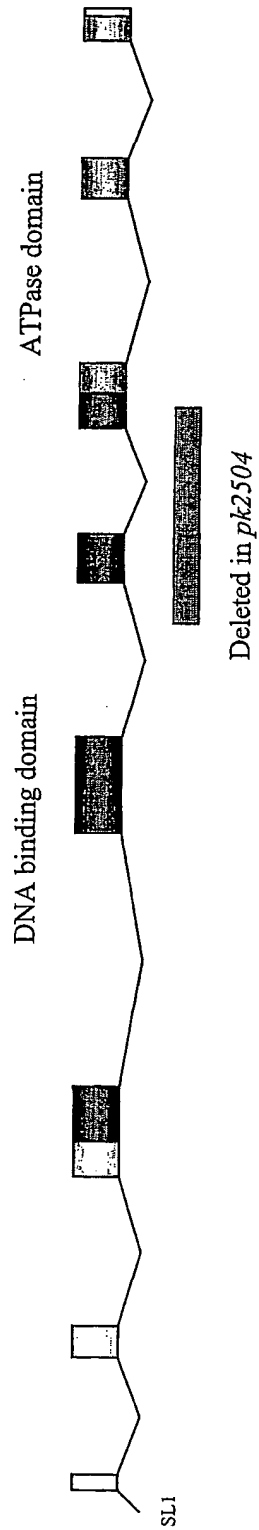


Fig. 1A

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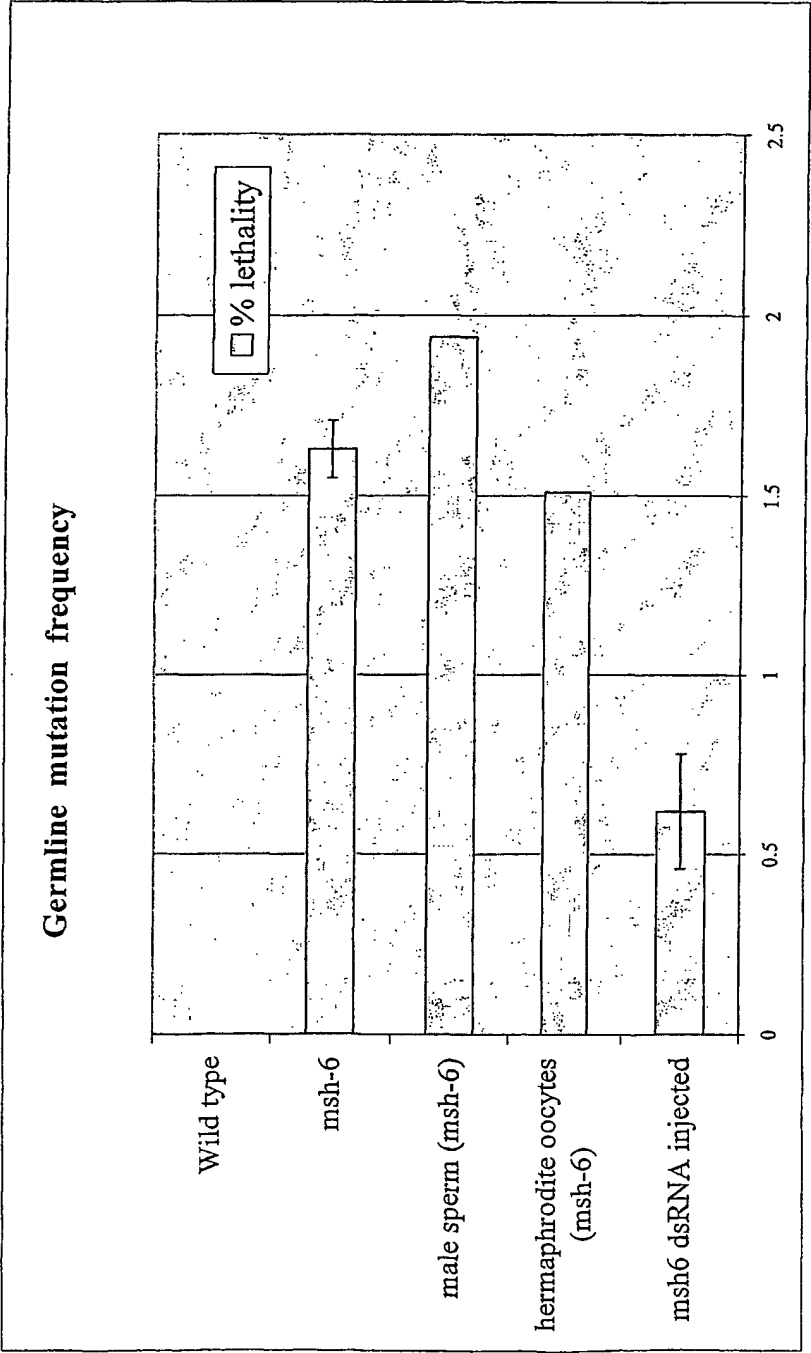
Docket No.: 2183-6201US

2/7

Fig. 1B

<i>C. elegans</i>	MSKROSSLSFFTNTPKSEKPEEEVKEKSVE-----EPKSLKNDTPKISNDS---051
Human	MSRQSTLYSFFPKSPALSDANKASARAREGGRAAAPEASPPGGDAAWSEAGPGRRLARSASP PKAKNLNGGLR 077
<i>S. cerevisiae</i>	MAPATPKTSKTAHFENGSTSSQKKKQSSLSFFSKQVPSG-----TPSKKVQKPTPATLENTATDKI 063
<i>C. elegans</i>	-----EKKVLRNSNKTVSSPVKTPRNAKRPKVWCSS 081
Human	RSVAPAAPTSCDFSPGDLVWAKMEGYPWVCLVYNHPFDGTFIREKGKSVRVHVQFFDDSPTRGWRLKPYTGSKSKEAQGGHFAKPEILRAMQ 177
<i>S. cerevisiae</i>	TKNPQGG-----KTGKLFVDVDENDLTAETVSTVRSDDIMHSQE 102
<i>C. elegans</i>	SEGEDDDGD-----EDFEMKEEHESSDESEADENASCEVESPESTPQSTPKRGGKKKISKPLLAENTP-----KSKVMKAKSKK 159
Human	RACALNKDKIKRLELAVCEPSEPEEEHEMEVGTTVYTKSEEDNEIESEEHVQPKTQGRSSRQKKRRVISDESIDIGSDVEFKPDTKEEGSSDE 277
<i>S. cerevisiae</i>	PQSDTMLNSN-----TTEPKSTTTDDELSSQSRNRHKRRVNYAESDDIDSTTFTAKKKKGKVDSESEDEYLPDKNDGDEDDDIADDEDKKE 194
<i>C. elegans</i>	VIP---DGEAVSMAG---VLDKMDKIMIEEG-E RRRIVEKTTGAKNKA VELEPAER-----FDHESFDFKPKDK 221
Human	ISSGVQDSEGLNSPVKVARNRKRMVINGSLKRKSSRKETPSATKQATSISETKNTLRAFSAPQNSAQHVSGGDDSSRPVWVYHETLEVLKEEK 377
<i>S. cerevisiae</i>	LAEDSCDDLLSLAETTSKKKFSYNTSHSSSPFTRNISRDNSKKKSRPNQAPSRYN-----PSHSQPSATSKSKKFNKQNEERYQVIVLE- 281
<i>C. elegans</i>	IRDGFKRPMSDBEYLPKTLWVPDFHCKQTEGHRWNTMSQHETTLLEKVGNEYETVHMDAVEVVRLNIAEMRE---SYAHAGFPEHRASKTAQDLA 318
Human	RREHRRSRPHDFDCASTLYVEEDFLNSCTEGMRKWQOLKSNQNFELAVICYKVGKEYELVHMDALIGVSEGLGVEMK---NWAHSGFPELAFGRYSDSLV 474
<i>S. cerevisiae</i>	-RDAQRSFKSDBEYLPRTLYTESAWNKETPEKQYHEITRSKMWECIVFFKKGKEEDLYEKDALLANALFOLKTAGGGRANMOLAGTEBMSFEYWAQFT 382
<i>C. elegans</i>	NHGKYVARIEETEPQMLEBNQKTKTK---EKVVRREVCRTVSNRRTYGVLDGVDLGSASSTLDPTAKHLLAIKEFHNPE---ETGKS-SYGVCHIDTT 411
Human	QKGYKVARVEGTETPEMMEARCRMAHISKYDRVVRREICRIITKGTQYSVLEGDPS-----ENYSKYLLSLKEEKED---SSGHTRAYGCVFVDTSE 564
<i>S. cerevisiae</i>	QMGKYVARVDQRESMAKEMRE---G-S-KGIVKRELOCILTSGLLDGDHLHSLDATTCLAIREEPGNFYNETQLDSSITVOKLNTKTEGAFTDTA 475
<i>C. elegans</i>	TAHIRIGOEEDDYRSQRLRLANVIVVQAIVERGSISSTTKSLING-ILFSVPVEHLPPKIDMTAEDVVRIVSNEEDYGSAS---EWBEVLKQML 505
Human	LKGFFIQGEPDRHCRRRTLVAHYPPVQVLEPKGNLSKETKTIILKS-SLSCSLQEGLLPGSDWDASKTLRTLLEEYEREKLSDGIGVMLPOVLKGMT 663
<i>S. cerevisiae</i>	TGELQMLEEDDSECTKLDTLMSQVRPMEVVMERNNSTLANKIVKENSAPNATNEVKAGEEYDCDKTYAEIISSEYFSTEED---WBEVLKSY 569
<i>C. elegans</i>	EDS-SILPKPSTDWQLALSAEGAIWYLRDLSLIEVMLSNRVTIYN---SNMENDQKKEKIDWNGKNLIDGTALLENLIVPNGRD-SHLSSTIYVIN 600
Human	SESDSIGLTPGEKS ELALSAEGGVFYLRKCLTDQELLSMANEEYVPLDSTVSPTRSGAIFTKAYQRWLDVAVTLNLEIFLNGTNGSTEGTLLEVD 763
<i>S. cerevisiae</i>	DTG-----KKGESBEGGLLYLWLKLEKNLISRNKIEKD-----FVKSQHSMLDGTTLQNLSEFNSNFGSDKGTLEKLFN 644
<i>C. elegans</i>	KCSIEFGRELLRSNLOETCDPKKLEQSKALKWLVSPDASSEMTTATATLKKIPDLORLQKTHITIGLKYSKHPDSRAIFDITKTNOKRIAEELAA 699
Human	TCHTPEKRELLKQDLCAPLCNHYAINDRLDAIEDLV---VPDKISEVELLKKLPDLERLLSKTHNVGSPKLSQNHPSRAIMYEETYSKKRIIDFLSA 860
<i>S. cerevisiae</i>	RAITPMCKRMMKRTMHELLKNDIESRLDSVDSLQD---ITLAEQLEITFSLPDLERMLARILSR-----TIRVKDFEKV 731
<i>C. elegans</i>	IDGKLCNKLKKEYIKVQKEGEGEILLDELLGNEQ-----MEEVDENIYFFERMEDRSTAMKDGKIVENAGOEYDEALNRVKBALNEINDYKDSVAK 795
Human	LEGKVMCKLIGIMEEVA-DEFKSKILKOVISLQTKNPEGRFPDLTVLELNWDTAEDHEKARKTGLITEKAGETSDYDQALADIRNEQSLLEYLEKQRN 960
<i>S. cerevisiae</i>	ITAGETIIELODSLKNNDLKEDVSKYISSFP-----EGLVEAVKSWTNAERQKALINENIIVEQRGEIEFFKSMDRIOBLEDEIMELMTYRK 808
<i>C. elegans</i>	KYSCS-IKEVDSCKVKVLLMBENTKVS-----SSFELKSRRGETRYSTPDSEQLVAAIDAVEKEKSKLGDATRVVEQEGHKN-PIWLETVKLVSS 887
Human	RIGCRTIVYWGIGRNRQLEHBNFTTR---NLPEEYELKSTKNGCKRYWTKTIEKLANIINAEERRDVSLKDCMRRLFYNGDKNY-KDQSAVECIAV 1056
<i>S. cerevisiae</i>	QFKCSNIOYKDSCKELVYTIETPISATKN-----VPSNIVQMAANATYKRYYSDEVRLARSMAEAKIEHKTLEEELKNBLCKQSDAHYNTIMPFTQATSN 904
<i>C. elegans</i>	FQVLTSLALFAKSSFFEMCEEDFNATDP-----YLIVDKGVHPCIALQSRNE---YTQITTSFIANSTTMGASEAAMVLGTGPNMGKSTLMRQTAV 977
Human	LQVLLCLANYSRGGDGMCREVILLPEDTPP-----FLELKGSRHPCITKTTFGDD-FIPNDILIGCEEEQENGKAYCVLVGTGPNMGKSTLMRQAGL 1149
<i>S. cerevisiae</i>	IDCLLATRTSEYLGAPSCEETIVDEVSKTNTQLNGFLKFKSLRHPCFNLGATTAKDFIPNDIELGKEQPRIG-----LLIGANAAKSTILRMACY 997
<i>C. elegans</i>	LAILAHGSMVPAFMRLTPIDRIFTRIGANLRIMCESTFFIEIKETDIMKNATKHSLLVDELGRGTSTFDGTAIASAVLQKISDDLACRTFFSTHY 1077
Human	LAVMAQMGCVPAEVCRLTPIDRVFTRIGASPRIMSESTFFVELSETASIMHATAHSIVVDELGRGTATFDGTAIANAVVKELNETIKRTLESTHY 1249
<i>S. cerevisiae</i>	AVIMQMGCVPCESAVLTPIDRIMTRIGANFNTHQKSTFFVELAETKKIDMATNRSLVDELGRGSSSDGFAIASVVLHVATHIQLSGFEATHY 1097
<i>C. elegans</i>	HSICDSFTNHPNVLAHMKCVVOKENEDPTMEDVTFELYELSESICPKSYGFIYAKIAGIDHQVVRNAYLESNKFASNLIIDPKIRHLVECARDNDFVG 1177
Human	HSLVEDYSQNVAVRIGHMCMVEN-ECEDPSQETITFLYKFIKACEKSYGQNAERLANLPEEVIQKGRKAREFEK---MNQSLRLEREVCLAS----- 1340
<i>S. cerevisiae</i>	GTLASSFKHHPQVSLKNSILVDE-----ATRNVTFLYKMLEQSEGEFGHVAHSMCGISKETIDNAQIAADNLEHTSLVKERDLAA NNINLEGVVSP 1191
<i>C. elegans</i>	ELKRMIEAI 1186
Human	-----
<i>S. cerevisiae</i>	GGQLQSDFVRITAYGDGLKNTKLSSGEGVLVYDWNIKRNVKLSLFSIIDDLQS 1242

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Spontaneous germline mutation frequency in wildtype *C. elegans*, *msh-6* genetic mutants and wildtype *C. elegans* exposed to *msh-6* dsRNA.

Fig. 2

4/7

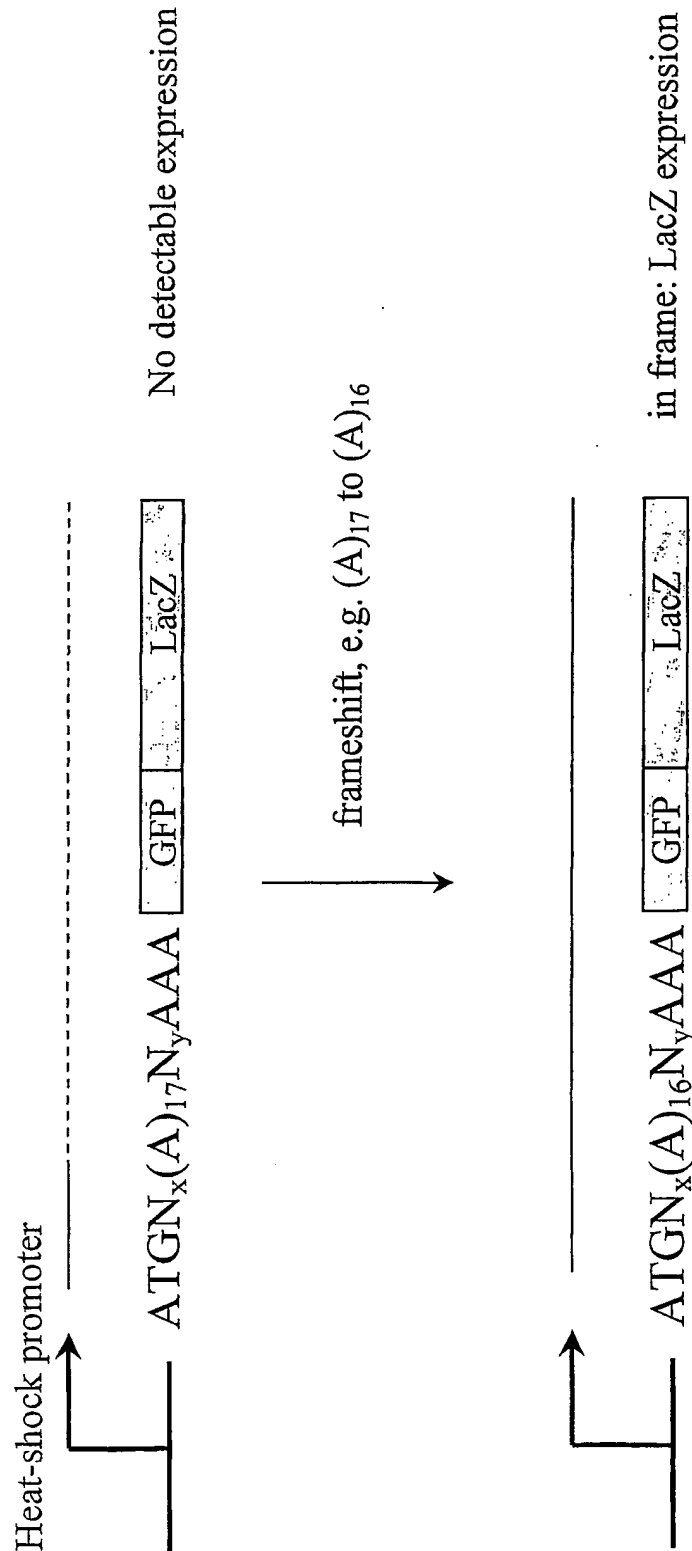


Fig. 3: Outline of the principle to detect somatic repeat instability

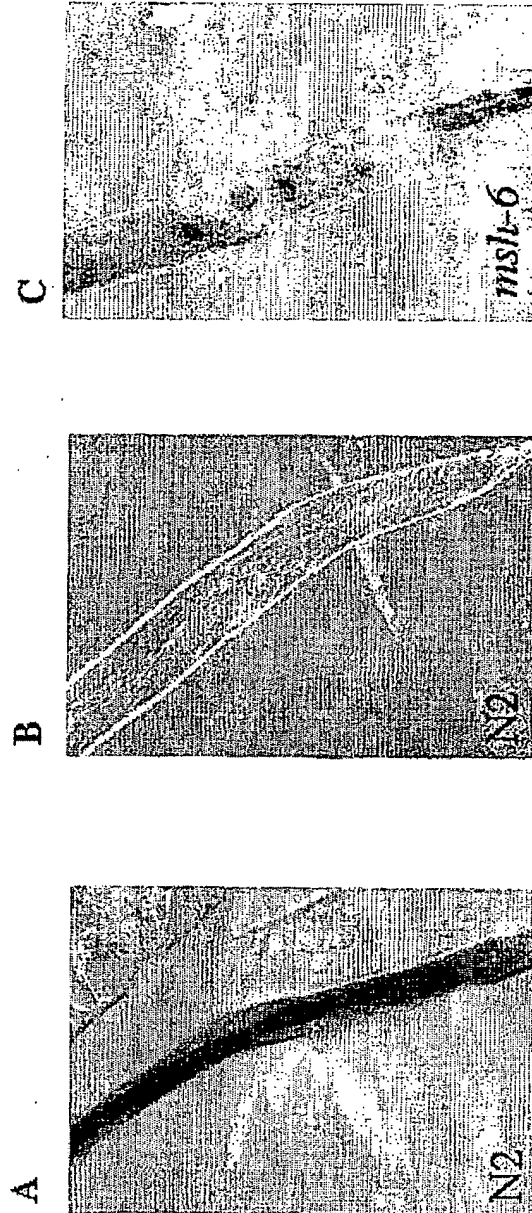


Figure 4: A) Wildtype *C.elegans* containing the in-frame construct, B) the +1 out of frame construct. C) Genetic *msh-6* mutants that contain the +1 out of frame construct display LacZ expression.

6/7

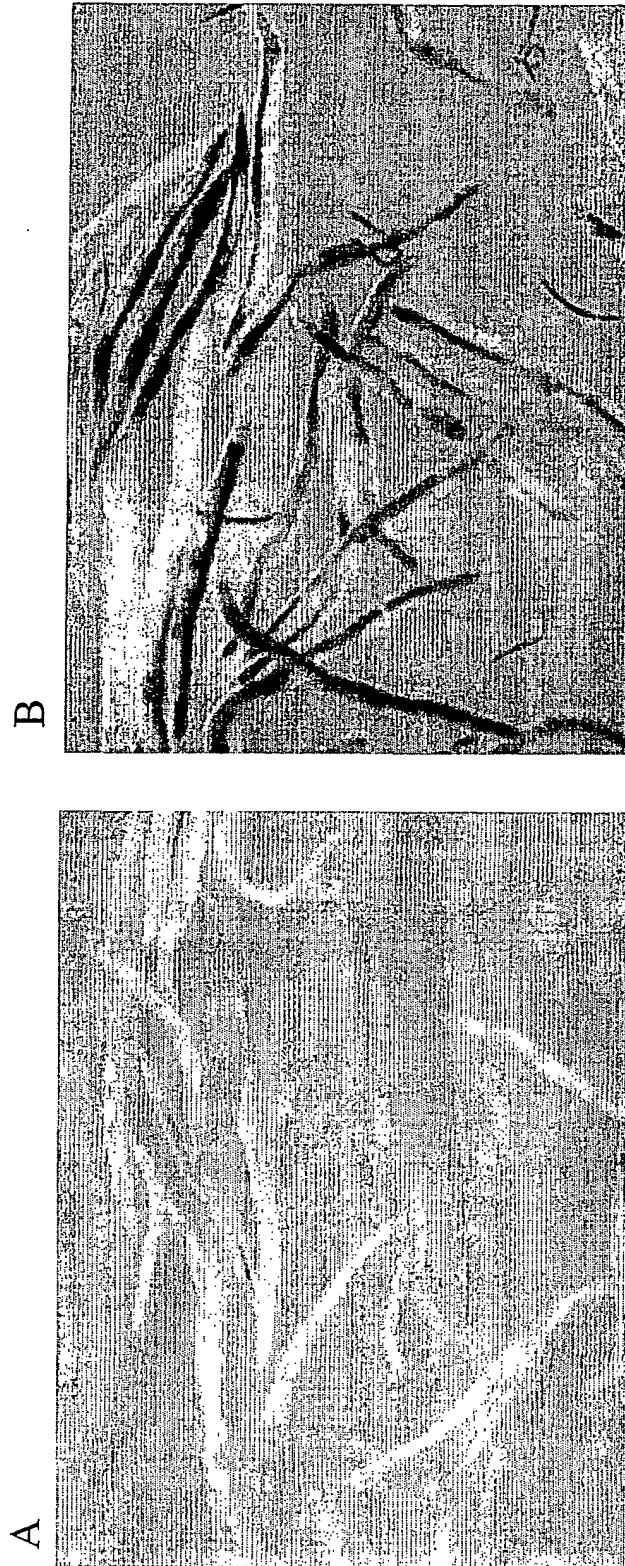


Fig 5: *C. elegans* populations fed on *E. coli* that produce dsRNA homologues to the *C. elegans* genes *unc-22* (A) and *msh-6* (B)

7/7

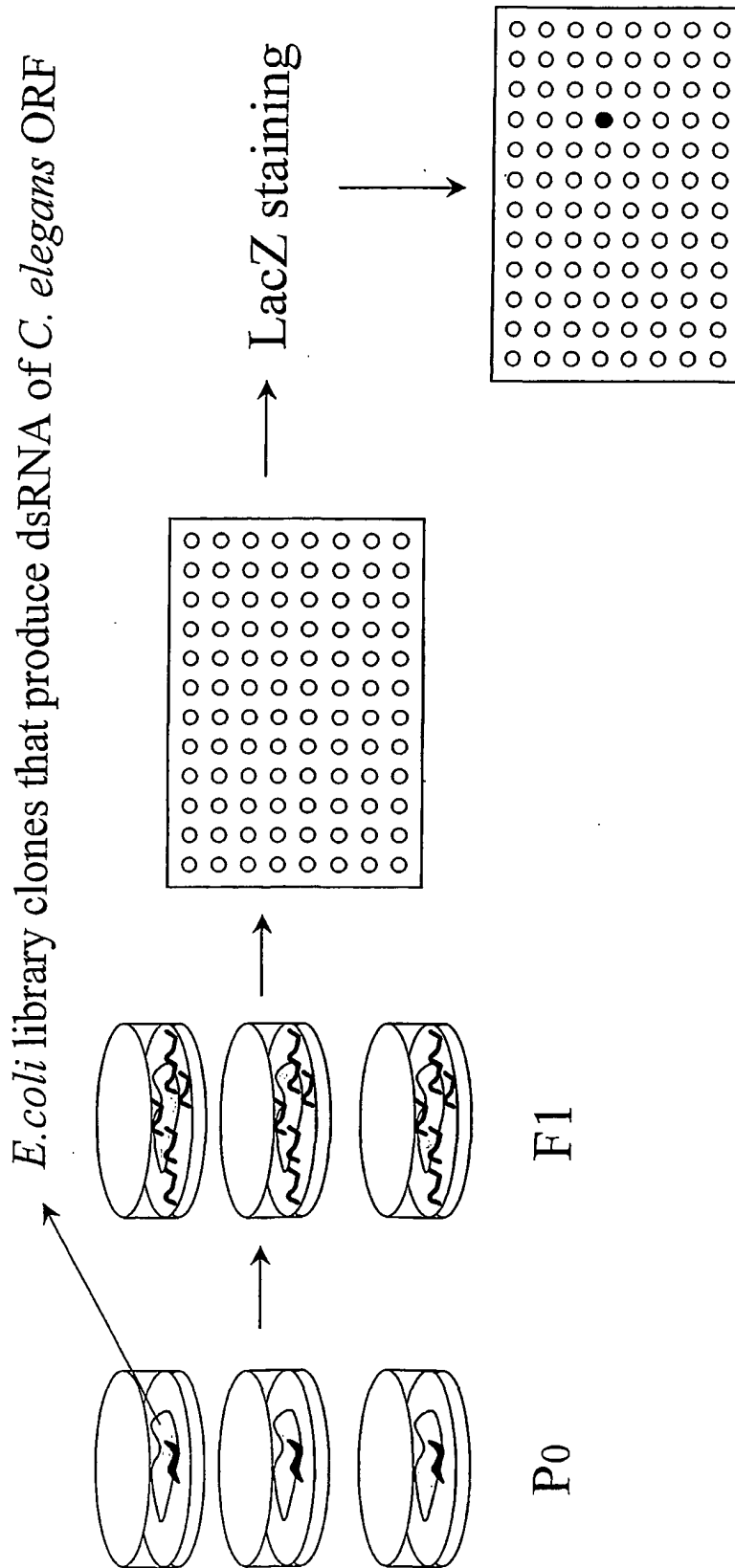


Fig. 6: Schematic representation of the high throughput RNAi based screens to identify novel mutator loci: Individual animals are fed on dsRNA producing bacteria, the progeny is collected and assayed for beta-galactosidase activity.